Vertebrate development: **Et in Arkadia**

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The similarities in organiser formation in *Xenopus* and mouse embryos have remained elusive. Recent evidence suggests a common mechanism, in which an intracellular protein, Arkadia, is required for formation of the mouse organiser and potentiates the effects of the signalling protein Nodal.

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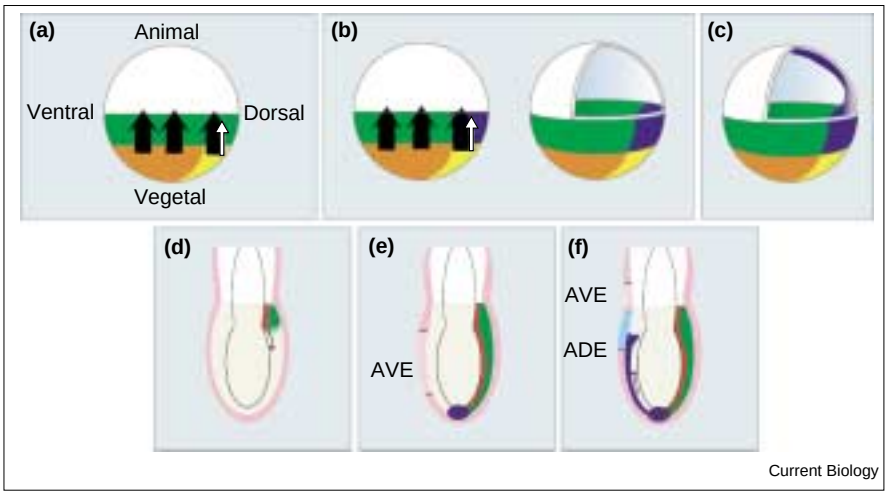
A critical point in the development of the vertebrate embryo is the formation of the organiser, variously referred to as Spemann's organiser in the frog *Xenopus laevis*, the shield in zebrafish, Hensen's node in chick and the node in mouse embryos. This specialised region of the early embryo is ultimately responsible for the generation of aspects of both dorso-ventral and antero-posterior polarity. Two recent papers [1,2] address organiser formation in mouse and *Xenopus* and shed light on the role of the organiser and the mechanism of its induction.

How similar is organiser formation in *Xenopus* and mouse? In the *Xenopus* embryo, Spemann's organiser forms in the dorsal marginal zone as a result of signals from the Nieuwkoop centre in the underlying vegetal hemisphere [3,4]. During gastrulation, signalling from the vegetal hemisphere induces a torus of mesoderm in the overlying marginal zone. The Nieuwkoop centre on the dorsal side of the embryo modifies these signals and specifies the dorso-anterior cell types of the organiser (Figure 1). Spemann's organiser regulates neural patterning and axis formation and differentiates into dorsal axial structures such as anterior endoderm, prechordal plate and notochord [3].

In mouse, gastrulation begins with the formation of the primitive streak, through which cells leave the epiblast layer to form embryonic mesoderm. The primitive streak extends towards the distal tip of the embryo where it forms a node (Figure 1). During this time extraembryonic tissues such as the visceral endoderm, which will not contribute to the embryo proper, regulate key aspects of embryonic regionalisation. Primitive streak formation is regulated by signals from extraembryonic tissues whilst the anterior visceral endoderm regulates the induction of anterior forebrain structures [5]. Thus comparison of *Xenopus* and mouse gastrulation raises many questions. What induces the mouse node? Does the mouse possess a Nieuwkoop centre? And what role does the node play in the general development of the embryo? Episkopou *et al.* [1] describe an insertional mutation in the mouse, *Arkadia*, whose analysis illuminates some of these questions.

Figure 1



Formation of the organiser and its derivatives in *Xenopus* and mouse embryos. In *Xenopus* embryos **(a–c)** signals from the vegetal hemisphere induce mesoderm in the marginal zone (green). These mesoderm inducing signals (black arrows) are modified by signals (white arrow) from the Nieuwkoop centre (yellow) resulting in organiser formation in the dorsal marginal zone (blue). Subsequently the organiser differentiates into tissues such as axial mesoderm lying at the dorsal midline. **(d–f)** The early mouse embryo comprises two principle tissue layers, the upper, epiblast layer which will form the embryo proper and the lower, extraembryonic, visceral endoderm (pink). The primitive streak (red) through which cells leave the epiblast to form embryonic mesoderm (green) begins at the posterior of the embryo and extends in an anterior direction towards the distal tip where the node forms (blue). Cells from the node region give rise to a variety of 'dorsal' tissues including axial mesoderm (dark blue) and anterior definitive endoderm (ADE, light blue) which displaces the anterior visceral endoderm, AVE.

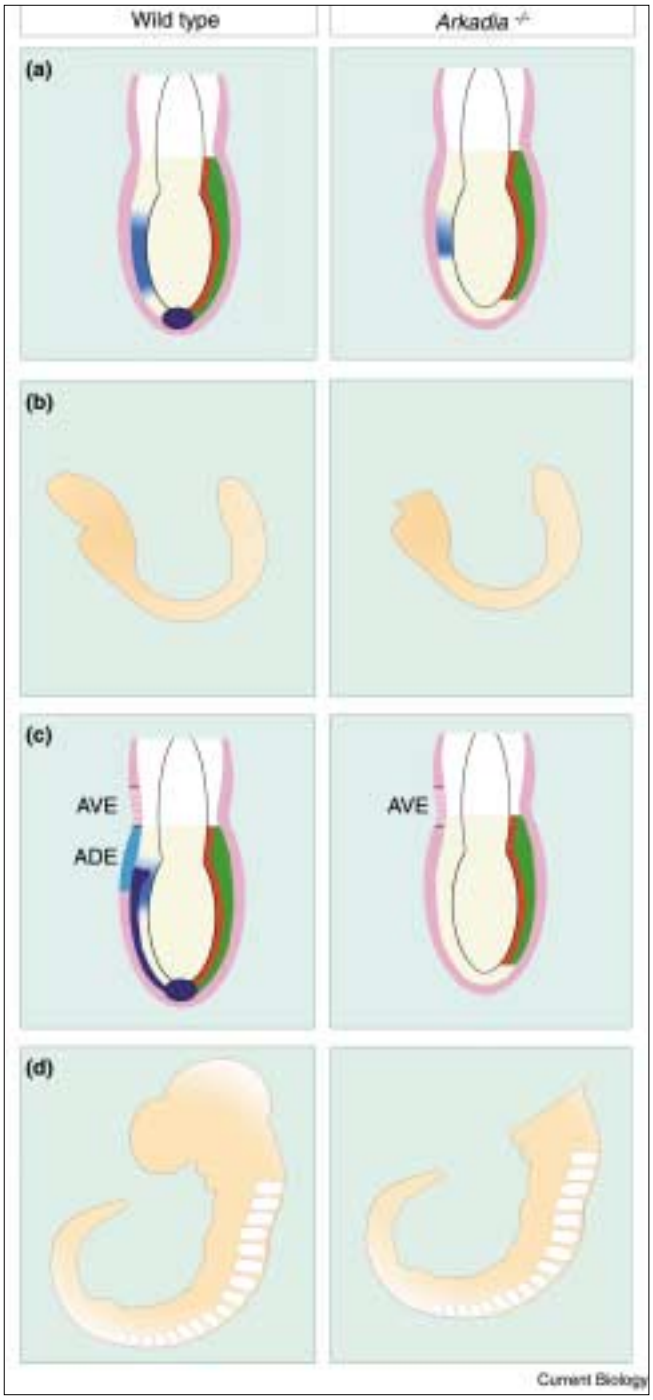
In embryos mutant for *Arkadia*, anterior structures such as midbrain and forebrain are lost by mid-neurula stages of development (Figure 2). Induction of the anterior forebrain depends on signals from the anterior visceral endoderm (AVE) whereas its maintenance requires signals from the anterior definitive endoderm (ADE) and prechordal plate (discussed in [6]). In *Arkadia* embryos, cells of the AVE form normally and are displaced anteriorly, as in wild type embryos. However, the ADE and ADE precursors that normally reside in the anterior primitive streak [7,8] are absent, at least as assessed by the analysis of regional markers; it remains to be seen whether these cells are indeed lost. It is plausible that the loss of forebrain structures in *Arkadia* embryos reflects the failure of formation of the ADE. Analysis of *Brachyury* and *Sonic Hedgehog* (*Shh*) expression in these embryos showed that the primitive streak forms normally, but axial mesoderm is absent. Analysis of *HNF3β*, a gene expressed in the node and its derivatives [9,10], showed that this phenotype was caused by the absence of node formation at the distal tip of the primitive streak. Thus the principal defect in *Arkadia* embryos is a failure to form the anterior primitive streak and node.

These data show that Arkadia has a specific role in the formation of the mouse organiser which is separable from other aspects of gastrulation, and are evidence of an activity in mice analogous to the Nieuwkoop centre. The ubiquitous expression of *Arkadia* RNA in early embryos does not point to an area in which localised Arkadia signalling might induce a node. However, an analysis of chimaeric embryos [1] showed that Arkadia is required only in extraembryonic tissues. Thus, embryos composed of mutant embryonic and wild-type extra-embryonic tissue have a wild-type phenotype, while chimaeras of wild-type embryonic and mutant extra-embryonic tissue show an *Arkadia* phenotype.

What is Arkadia's function in mouse organiser formation? Episkopou *et al.* [1] and Niederlånder *et al.* [2] show that Arkadia is an intracellular protein with two putative nuclear localisation signals and a carboxy terminal RING-H2 finger motif — a zinc-binding protein–protein interaction domain which may also target proteins to the proteasome. Therefore Arkadia is not a direct signal but instead must interact with one.

Episkopou and colleagues [1] investigated a potential interaction between *Arkadia* and the mesoderm inducer, *Nodal*. Mouse *Nodal* mutants lack a primitive streak and show severe deficits in mesoderm formation [11]. Mice heterozygous for either *Nodal* or *Arkadia* loss-of function mutations have no phenotype. However mice heterozygous for both *Nodal* and *Arkadia* mutations show a phenotype similar to homozygous *Arkadia* mutants. This suggests that Arkadia normally acts with Nodal signalling to induce the formation of the mouse organiser. Although the

Figure 2



The *Arkadia* mutation results in degeneration of anterior structures due to loss of node derived tissue. (a) Anterior markers (blue) are initially expressed in *Arkadia* mutant embryos indicating the induction of anterior tissues. (b) Subsequently anterior brain structures degenerate and are completely absent anterior to the hindbrain by 9.5 days of development. (c) Closer analysis reveals that although primitive streak formation occurs normally, the node and anterior definitive endoderm do not form, resulting in a failure to maintain anterior structures (d). See Figure 1 for key to tissues.

mechanism for such a potential interaction remains unclear, parallel studies in *Xenopus* [2] provide clearer evidence that Arkadia is able to modulate Nodal signalling.

In *Xenopus* embryos, the highest levels of *Arkadia* RNA are restricted to the presumptive dorsal region of the blastula embryo, a region which includes both the Nieuwkoop centre and the future organiser. Furthermore, experiments suggest that Arkadia can induce characteristics of Spemann's organiser [2]. Misexpression of *Arkadia* in cells of the ventral marginal zone induced ectopic dorsal characteristics in a dose-dependent manner. In *Xenopus*, as in the mouse, Arkadia does not act as a dorsal mesoderm inducer: misexpression of *Arkadia* in animal cap tissue did not induce mesodermal or organiser-like characteristics [2]. The data suggest that Arkadia alters the response of marginal zone cells to signals already present in this tissue and may be a 'dorsal modifier'.

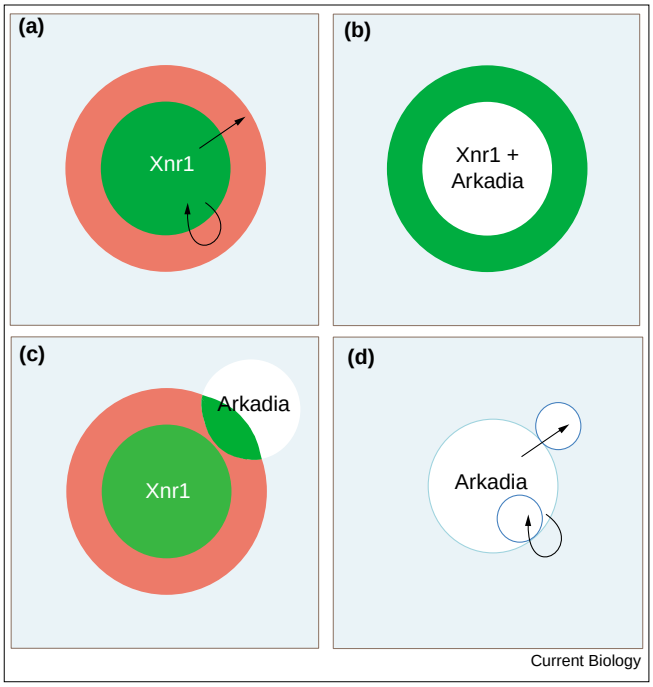
What endogenous signals could be modified by Arkadia to promote dorsal mesoderm formation? Arkadia does not antagonise the ventralising effects of BMP4. However, Niederlånder and colleagues [2] observed that Arkadia was able to potentiate the effects of mesoderm inducers of the Nodal/Activin family when co-expressed. Conversely, the dorsalizing effect of Arkadia in ventral marginal tissue was abolished by co-expression of the Nodal antagonist Cer-S. These observations clearly strengthen the assertion by Episkopou and colleagues [1] that Arkadia regulates organiser formation by modulating Nodal signalling.

Nodal/Activin signals are dose-dependent inducers of mesodermal cell types [12–14] that can pattern the organiser region by acting as morphogens [15,16]. Thus Arkadia may act to steepen a Nodal concentration gradient in the forming organiser region of the embryo. If this is the case, how might Arkadia do this? Nodal signalling can be mimicked by misexpressing activated forms of the receptor Alk4 or a downstream component of the Nodal signalling pathway, Smad2. Interestingly, Arkadia does not potentiate the mesoderm-inducing activities of either of these downstream effectors [2], and is therefore not directly downstream of the Nodal receptor. Arkadia could sensitise the Nodal receptor, it could regulate a cofactor or inhibitor of Nodal signalling [17], or it could affect the production of the Nodal ligand itself. Some of these possibilities predict that Arkadia could function in cells receiving a Nodal signal whilst others imply that Arkadia potentiates the Nodal signal in the cells which produce it. Experiments in which *Arkadia* is expressed either in cells producing the Nodal family member Xnr-1 or in cells adjacent to them suggest that both predictions may be true.

Misexpression of *Xnr-1* in *Xenopus* animal blastomeres induces two marker genes, *Gsc* and *Brachyury* in a dose-dependent manner. *Gsc* is expressed within the *Xnr-1* expressing cells, which are presumed to experience the highest levels of Xnr-1 protein. *Brachyury* is induced only at lower levels of Xnr-1 and is expressed in a ring of cells around the *Gsc* domain (Figure 3). Co-expression of *Arkadia* and *Xnr-1* fails to induce *Brachyury*; *Gsc* is no longer induced in the cells expressing Xnr-1 but is expressed instead by the cells which would express *Brachyury* in response to Xnr-1 alone. Thus, Arkadia potentiates the response of cells to a Nodal signal in a non- cell-autonomous manner. However, it is only able to exert this effect over a short distance since *Brachyury* expression is not shifted in a manner similar to *Gsc*. This may reflect a restriction of an extracellular cofactor regulated by Arkadia or instead point to the relative distance over which Nodal itself is able to act [16].

When Arkadia is expressed in cells adjacent to a Xnr-1 expressing clone, Niederlånder and colleagues [2] found

Figure 3



Arkadia potentiates Nodal signals. **(a)** When misexpressed in animal cap cells, the *Xenopus Nodal* gene *Xnr1* induces the expression of the dorsal markers *Gsc* and *Xbra* (*Brachyury*) in an apparently dose dependent way. Cells within the *Xnr1* expressing clone express *Gsc* (green) whilst those adjacent to it express *Xbra* (red). **(b)** Coexpressing *Xnr1* and *Arkadia* results in suppression of *Xbra* and a shift in *Gsc* expression from within the clone to those cells adjacent to it. **(c)** When *Arkadia* is expressed in a clone of cells adjacent to an *Xnr1* expressing clone, *Xbra* is downregulated and *Gsc* induced within the *Arkadia* expressing cells. **(d)** Arkadia may function by altering the extracellular environment to bring about a locally enhanced 'field' within which the effects of Nodal are potentiated.

that *Brachyury* is suppressed, as in cells co-expressing Xnr-1 and Arkadia. And subsequent experiments have demonstrated that in this situation *Gsc* is induced in the Arkadia expressing cells, suggesting that Arkadia potentiates the response to Nodal in these cells (M. Jones, personal communication). Whilst the mechanism of its action is unclear, Arkadia may generate a locally potentiated 'field' for Nodal signalling by bringing about a change in the extracellular environment.

Can the data from mouse and *Xenopus* be combined in a single model of organiser formation? The increased expression of *Arkadia* in the presumptive dorsal region of the *Xenopus* blastula may enhance Nodal signalling in this domain and lead to the formation of the organiser. In mouse, however, enhanced expression of *Arkadia* RNA has not been demonstrated in the region where the node forms and it is difficult to see exactly when and where Arkadia might exert its effect. And the distribution of Nodal in the early mouse embryo provides few further clues. Nodal is expressed in both epiblast and visceral endoderm during gastrulation and it has yet to be shown where Nodal is required during node formation. It is important to note that Arkadia and Nodal may not necessarily act in the same tissues to bring about node formation but instead form components of a feedback interaction between the extraembryonic tissues and epiblast. Such a possibility is consistent with the notion that Arkadia provides a local, non cell-autonomous potentiation of Nodal signalling.

It has been shown that subpopulations of cells, including cells expressing early markers of the organiser, are found within the primitive streak and are later resolved as a morphological node forms [5]. It is possible then that the Nieuwkoop centre-like activity mediated by Arkadia may occur at an earlier point in gastrulation and affect cells within the elongating primitive streak or even the epiblast itself. In the chick it has been demonstrated that the organiser, Hensen's node, is in fact a transient cellular state which cells assume as they move through this specialised region [18]. In this situation, a region of the primitive streak has been ascribed properties analogous to those of the Nieuwkoop centre. It is therefore conceivable that in the mouse enhanced Nodal signalling, as a result of Arkadia, functions at an earlier time to bring about the formation of such a signalling centre in the primitive streak. It might be possible, using tissue specific promoters to perform localised cell ablations or tissue specific knockouts, to determine exactly where and by what mechanism the organiser is induced in the mouse. These are far from trivial experiments however, and it may be some time before we fully understand this complex process. Whilst Arkadia is pointing us in the right direction we have further to go to reach the promised land.

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